United States Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service

FGIS Issuance Change

CHANGE TO	☐ DIRECTIVE	☐ MANUAL	☒ HANDBOOK

CHANGE NO:	TO *(No.)*	TITLE:	DATE:
9		Aflatoxin Handbook	8-30-04

PURPOSE OF CHANGE: The Aflatoxin Handbook has been revised to clarify the procedures for performing a supplemental aflatoxin analysis using the RIDASCREEN® Fast Aflatoxin test method, and to make minor editorial changes.

FILING INSTRUCTIONS

Remove	Dated	Insert	Dated
Page 6-5	1-5-04	Page 6-5	8-30-04
Page 6-6	No date	Page 6-6	No date
Page 8-9	6-24-02	Page 8-9	8-30-04
Page 8-10	No date	Page 8-10	No date
Page 10-3	3-4-02	Page 10-3	3-4-02
Page 10-4	No date	Page 10-4	8-30-04

Retain this issuance sheet as an aid in verifying handbook contents.

/s/ David Orr

David Orr, Director
Field Management Division

Distribution: A, C, E

Originating Office: PPB, FMD

6.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 50 ppb are reported as >50 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

6.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 50 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 50 ppb, the sample extract must be diluted so that a value between 5 and 50 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 70 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 1 ml of the original diluted filtrate (obtained from step e., section 6.3) with 1 ml of distilled/deionized water. The total volume is 2 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 34 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 68 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

True Aflatoxin Value = (10 ÷ 5) x 34 ppb
= 2 x 34 ppb = 68 ppb

6.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

6.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the syringe into a plastic garbage bag for disposal.

(4) Supplemental Analysis.

To determine and report an aflatoxin level higher than 300 ppb, the filtered test sample extract must be diluted so that a value between 5 ppb and 300 ppb is obtained. The final aflatoxin concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

(a) Using an Eppendorf pipette, add 0.5 ml (instead of 1.0 ml) of the filtered diluted extract to the top of the Aflatest column headspace. (See section 8.5 a (2) (b).)

(b) Analyze the filtered extract as a normal sample.

(c) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 240 ppb was the sample value obtained using the diluted test sample procedure, the actual concentration in the original sample was 480 ppb.

Example:	Diluted test sample extract result	240 ppb
	Dilution factor	x 2
	Actual aflatoxin concentration	480 ppb

Note: Laboratories may dilute samples as a first step if levels typically observed exceed 300 ppb and the applicant requests certified results above the range of the test kit.

b. Procedures for Testing Corn Germ Meal and Wheat.

(1) Extraction.

(a) Place 50 g of ground sample into blender jar.

(b) Add 10 grams of analytical, USP grade sodium chloride (NaCl) or food grade un-iodized salt.

(c) Add 200 ml of the 80/20 methanol/water extraction solution.

(d) Cover jar and blend at high speed for 1 minute.

(e) Remove the cover and pour the extract into a filter paper (Whatman 2V folded or S&S 591 24 cm pleated or equivalent) supported in a clean funnel.

(f) Collect the filtrate in a clean beaker labeled with the sample identification.

(g) After collecting approximately 25 ml of extract, carefully dispose of the filter paper and its contents.

(h) Pipette 5 ml of filtered extract into a clean beaker.

Note: If the solution filtration is slow (i.e., more than two minutes are required to collect 5 ml of filtrate), withdraw 5.0 ml of the clearest liquid from the top of the material held in the funnel (see step (e) above) and transfer it to a clean container.

(i) Add 10 ml of distilled/deionized water and mix thoroughly.

(j) Filter the diluted extract through a glass microfibre filter (Vicam Cat. # 31955) supported by a small, clean funnel. Fold the glass microfibre filter gently without making a sharp crease to avoid breaking the glass microfibre filter.

(k) Immediately proceed with the Aflatest Affinity Column procedure.

Note: If this diluted filtrate turns cloudy, refilter using a new glass microfibre filter before proceeding with the analysis.

(2) Affinity Column.

(a) Prepare an Aflatest-P affinity column for use by removing both end caps and gently shaking the buffer solution from the top of the column.

(b) Using an Eppendorf pipette, add 1.0 ml of the filtered dilute extract to the top of the Aflatest column.

(4) Place 100 µl of conjugate (blue-labeled bottle) into each mixing well using a 100 µl pipettor with a new tip. Prime the pipette tip first before dispensing the 100 µl. Discard the pipette tip.

NOTE: "Prime the pipette tip" is accomplished by drawing liquid up into the tip and dispensing it back into the bottle once or twice.

(5) Place 100 µl of control (yellow-labeled bottle) into the first mixing well labeled "1." Prime the tip before dispensing. If testing more than one sample, also place 100 µl of control into mixing well #4 for the second sample, mixing well #7 for the third sample, and mixing well #10 for the fourth sample. Discard the pipette tip.

(6) Place 100 µl of sample each in mixing wells #2 and #3. Prime the tip first before dispensing. Discard the tip. Subsequent samples should be placed in wells #5 and #6, then #8 and #9, and then #11 and #12.

See the diagram below for an example of the procedure.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
mixing wells	O	O	O	O	O	O	O	O	O	O	O	O
	C	S1	S1	C	S2	S2	C	S3	S3	C	S4	S4

"W" = well number (e.g., #1 through #12)
"C" = control
"S1, S2, S3, & S4" = sample numbers

(7) Using the 12 channel pipettor and the overfill method (see note below), mix the contents of the mixing wells by pipetting up and down in the tips 5 times.

NOTE: The "overfill method" is performed by drawing greater than 100 µl into the pipette tips by pressing the pipettor to the second stop before placing tips into the solution. Place tips into the liquid and release the plunger slowly and completely.

(8) Transfer 100 µl to the antibody coated wells (the unmarked, clear wells). To dispense only 100 µl, press plunger to the first stop.

(9) Mix in the antibody coated wells by gently sliding the microwell holder back and forth on a horizontal surface for 15 seconds. Be careful not to allow solution to splash out of wells.

(10) Immediately following mixing, incubate for 5 minutes. Discard all mixing (red marked) wells and tips.

(11) With a wash bottle containing deionized/distilled water, fill each antibody well and dump the contents into a waste receptacle. Repeat this step five times.

(12) Turn microwell holder, with wells in it, upside down on a paper towel and tap gently until water is removed from the wells.

(13) Using the 12 channel pipettor and the overfill method, place 100 µl of substrate into each well.

(14) Mix gently by sliding the microwell holder back and forth for 15 seconds on a horizontal surface for 15 seconds. Be careful not to allow solution to splash out of wells.

(15) Immediately following mixing, incubate for 5 minutes.

(16) Discharge the remaining substrate in the pipette tips by plunging once or twice without drawing any additional liquid up into the tips. Save these tips for the next step.

(17) Using the 12 channel pipettor and the overfill method, add 100 µl of the red stop solution (red labeled bottle) into each well.

(18) Mix gently by sliding the microwell holder back and forth for 15 seconds. Again be careful not to lose any solution from the wells. Visually check the appearance of the wells. Discard all pipette tips.

(19) Read in a Bio Tek EL 301microwell reader using a 650 nm filter within 5 minutes of the addition of the red stop solution.